REMARKS

Entry of the foregoing, reexamination and reconsideration of the subject application, as amended, pursuant to and consistent with 37 C.F.R. §1.116, are respectfully requested in light of the remarks which follow.

I. Claim Amendments

By the foregoing amendment, claims 1, 4, 9, 10, 13, 18, and 19 have amended, and claims 5, 6, 14 and 15 have been canceled.

In particular, claim 1 has been amended to recite that "an increase of the thermal and optionally kinetic energy of said magnetically susceptible particles causes the formation of *temporary* pores in said biological membrane-enveloped structures." Support for this amendment can be found at least at page 6, lines 17-19 of the specification.

Claims 4 and 13 have been amended by deleting the phrase "the direction of said alternating gradient being generated by two coils and said sample is inserted between the coils" from both claims.

Claims 10 and 19 have been amended by deleting the phrase "and/or metabolism."

Claims 9 and 18 have been amended to recite "biological membrane-enveloped structures" rather than "cells." Similarly, claims 10 and 19 have been amended to recite "biological membrane-enveloped structures" rather than "host cells." These amendments are supported at least by original claim 1.

The amendments to the claims have been made without prejudice or disclaimer to any subject matter canceled or recited herein. Applicants reserve the right to file at least one continuation and/or divisional application directed to any canceled subject matter. No new matter has been added, and entry of the foregoing amendments of the above-identified application are respectfully requested.

II. Response to Claim Rejections Under 35 U.S.C. § 102

Claims 1-3, 7, 9, 10, 12, 16, 18 and 19 have been rejected under 35 U.S.C. 102(b) as allegedly being anticipated by U.S. Patent 4,889,120 (Gordon). This rejection is respectfully traversed.

It is well established that for prior art to be anticipatory, every element of the claimed invention must be disclosed in a single item of prior art in the form literally defined in the

claim. *See, e.g., Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 213 U.S.P.Q. 81, 90 (Fed. Cir. 1986). Applicants submit that Gordon fails to satisfy this requirement, for at least the following reasons.

First, Applicants submit that a blood vessel is not a "biological membrane-enveloped structure" as recited in the present claims. In particular, contrary to the Examiner's assertion, a "vessel or an artery vein" cannot be considered an "organelle at a subcellular level." Instead, a blood vessel or a vein is a tissue built up of millions of cells of varying kind in a tissue-specific arrangement of many layers of cells and intracellular space. A vein or blood vessel can also be described as an organ or part of an organ within a mammalian body.

On the other hand, the term "organelle at a subcellular level" or "subcellular organelle" is an accepted term in cell biology and introduced in basic undergraduate biochemistry and biology studies (*see, e.g.*, pages 15-22 in "Biochemistry" written by Christopher Mathews and K.E. van Holde, Oregon State University in 1990, published by Benjamin/Cummings Publishing Company). A subcellular organelle is by definition a small structure, enclosed by a lipid membrane, and located within a cell. Examples of subcellular organelles are cell nuclei, mithochondria, endosomes, lyzosomes, caveosomes, peroxisomes, vacuoles, autophagosomes, plastides, golgi, endoplasmatic reticulum and chloroplasts. Thus, a person of ordinary skill in the art would readily agree that a blood vessel cannot by any definition be described as a subcellular organelle.

Second, to expedite prosecution and not to acquiesce to the Examiner's rejection, claim 1, as noted above, has been amended to recite that the pores formed in the biological membrane-enveloped structures of the present invention are *temporary* pores. However, Gordon does not teach a method relating to temporary pores as recited in the present claims. In fact, the fusion disclosed in Gordon, for connecting veins or arteries, is completely different from the method of the present invention, wherein temporary pores are formed.

The method described in the present application and recited in the present claims is directed to the transfer of exogenic bioparticles (*e.g.* DNA) into (or out of) biological membrane-enveloped structures such as cells. The present method generates small temporary destabilisations of the cell membrane thus creating small, temporary openings or pores in the biological membrane-enveloped structures (*see, e.g.*, page 6, lines 13-24 of the specification). In this method, the temporary pores are open to the extracellular medium, allowing uptake of exogenic bioparticles from the environment outside the cell. For example, uptake of DNA is

promoted by letting the magnetic particles transport the DNA molecule on their surface into the target cells. Furthermore, several magnetic particles will bind to the cell surface, and each and every one of them will heat a nanovolume of the lipidbilayer of the cells, causing temporary pores in the membrane of the biological structures. Each magnetic particle will potentially give rise to a transport process of bioparticles across the membrane in very close vicinity to each magnetic particle. Furthermore, using the method described in the present application, each individual cell can be repeatedly treated with a higher dose of magnetic particles until the frequency and number of transient pores causes cell lysis of all cells in the sample, which is useful for killing cells and extracting intracellular components.

In contrast to the present invention, the methods of Gordon are directed to cell fusion. Cell fusion can be described as a sequence of interrelated events. Firstly, two cell membranes have to be brought very close together. Gordon achieves this by pulling the two magnetic particle-injected edges of the tissues (vessel or vein) into close proximity using a permanent magnet. According to Gordon, the magnetic particles are injected into or applied at the edge of the vessel or vein (see col. 5, l. 13-15 and l. 26-30) and spread evenly in all layers of the vessel wall. When the two veins or vessels are brought close together, the edges of the two vessels or veins come as close as needed to induce cell fusion at a cellular level (col. 7, lines 46-48).

A person skilled in the art knows that cell fusion, as taught in Gordon, is the merger of two cells into one cell. In scientific publications, heat has been proposed to promote the latter process of pore opening between the two bilayers, and Gordon describes a process wherein heat is generated in the magnetic material via the particles when they are exposed to an alternating magnetic field. However, it is clear that the magnetic particles of Gordon do not contribute to the molecular fusion process in any way other than by promoting heat. In addition, the particles that generate heat are located in the area where cell fusion takes place, due to the permanent magnet that attracts the magnetic particles within the two veins or vessels to the coil where the alternating magnetic field is generated. Thus, Gordon does not teach or suggest that heat is generated at the surface of each individual cell. In fact, from a biological and biophysical point of view it is obvious that the particles cannot be situated on the membrane surfaces that constitute the area of fusion, since that would inhibit any cell fusion process.

In contrast to the temporary pores of the present invention, a fusion pore is a

permanent opening between two cells. In particular, the two cells are opened to each other and their intracellular components are mixed in one novel cell cytosolic compartment. No material from the outside of the membrane will end up in the newly formed fused cell. The (single) fusion pore is stabilized and the pore grows and finally the opening between the two cells are total and one cell is formed from the two original cells. A fusion pore is thus a permanent opening formed *between the two cells*, and after the cell fusion the two original cells will not exist anymore. Thereafter, it is possible for other epithel cells to grow and divide across the bridge that the fused cells have created such that the two veins or vessels are joined together. Accordingly, while pore formation may be a part of the cell fusion process, the fusion pore of Gordon is permanent and it is not a temporary pore that is opened to an extracellular compartment. Thus, in marked contrast to the present invention, the pore in Gordon cannot result in the uptake of any exogenic material from outside the cells. Furthermore, it would be devastating for the cells undergoing cell fusion if the fusion process did induce permanent pores open to the exterior of the cells, since this would cause cell death (lysis) and no fusion.

Finally, Applicants submit that if a person were to attempt to introduce exogenic material into the cells using the method disclosed in Gordon, the attempt would certainly fail. After adding exogenous DNA and magnetic particles of the kind that Gordon describes to the cell culture, the magnetic particles would be attracted by a permanent magnet to the centre of a coil that can generate an alternating magnetic field. If the magnetic particles were not attached to the cell surface the particles would end up on the magnet in the center of the coil and the cells would be unaffected. Thus, no treatment would occur and no cell fusion will be initiated. However, if the magnetic particles were attached via an antibody or receptor ligand to each cell in the sample the cells would be pulled together like a pellet on the permanent magnet. The added DNA molecules would be found in the surrounding extracellular medium. If the magnetic particles were heated in a successful way, without killing the cells due to overheating them or due to the pellet formation in such concentrated volume, the very best outcome would be fused cells. These fused cells would not carry any extracellular fluid in accordance with the process of cell fusion and hence would not carry exogenic DNA. Thus, the method of Gordon could never result in the effect shown in the present specification and recited in the present claims.

In conclusion, Gordon does not disclose, either explicitly or inherently, a method that

allows for the introduction or extraction of exogenic bioparticles into or from biological membrane-enveloped structures. Furthermore, Gordon does not disclose any event that promotes temporary pores in a biological membrane.

Since each and every element of Applicant's claimed invention is not taught, either explicitly or inherently, by Gordon, such reference fails to anticipate the claims of the present application. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

III. Response to Claim Rejections Under 35 U.S.C. § 103

Claims 4-6 and 13-15 were rejected under 35 U.S.C. 103(a) as allegedly being unpatentable over U.S. Patent 4,889,120 (Gordon) in view of U.S. Patent 6,149,576 (Gray et al.). This rejection is respectfully traversed, for at least the following reasons.

As discussed above, Gordon does not teach or suggest the methods recited in the present claims. In addition, Gray et al. fails to remedy the serious deficiencies of Gordon. Therefore, even if Gordon was combined with Gray et al., one of skill in the art would still not arrive at Applicants' claimed invention.

Since a proper *prima facie case* of obviousness has not been established, withdrawal of this rejection is respectfully requested.

CONCLUSION

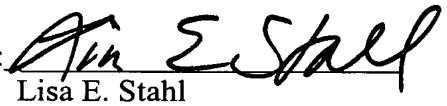
In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions relating to this Amendment and Reply, or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney concerning such questions so that prosecution of this application may be expedited.

Respectfully submitted,

BUCHANAN INGERSOLL PC

Date: July 5, 2007

By: 
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